

## Bioassay Test for Auxinic Herbicide Residues in Compost: Protocol for Gardeners in Washington State

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### Introduction

Effects of aminopyralid residues in dairy organic matter (manure, composted manure, silage) applied to broadleaf crops on farms and gardens in Whatcom County have been seen in 2009 and 2010. Aminopyralid is an auxinic herbicide that will cause damage to sensitive broadleaf plants such as tomato, beans and peas; these plants will usually not die, but will produce no or few, low quality fruit. Aminopyralid is a broadleaf herbicide used on grassland and rangeland.

Aminopyralid was applied to fields for cut forage of several dairies in Whatcom County. The dairy farmers were unaware of issues that may result from using the resulting manure as organic matter on farms or gardens growing certain crops. When this manure was exported from the dairy, the farmers did not realize that the product may result in damage to crops.

Other herbicides that may be found in organic matter, such as clopyralid, triclopyr, 2,4-D, and dicamba, will cause similar symptoms to those of aminopyralid. This bioassay will determine if an auxinic herbicide is present, but not which one.

### Purpose of this protocol

This protocol is intended to answer the following question using simple, relatively inexpensive procedures:  
*Will this test material (compost, manure, or topsoil blend) cause plant growth symptoms consistent with auxinic herbicide damage in susceptible plants?*

### Using the Bioassay – General Procedures

This bioassay is intended for use by homeowners and gardeners. It has been modeled after the bioassay developed by Washington State University and Washington State Department of Ecology in 2002 (<http://www.puyallup.wsu.edu/soilmgmt/Pubs/CloBioassay.pdf>).

In this protocol, peas are planted and allowed to grow for two to three weeks until three sets of leaves have appeared. The plants are compared to control plants grown at the same time and evaluated for herbicide damage.

Photographs of plants grown in compost known-to-be contaminated by aminopyralid are available in Figures 1 and 2 as an aid to identifying plant damage consistent with auxinic herbicide damage.

### Materials

- Test material (compost, manure, or topsoil)
- Potting mix (compost-free; peat-based commercial mix with fertilizer included)
- 4" plastic pots (new, manufacturer not specified; volume = 0.75 liters)
- Plastic saucers
- Garden pea seeds (variety not specified)
- Plastic bags
- Disposable gloves

## Specific Procedures

1. Evaluate test material (compost, manure, topsoil mix)
  - Record observations of odor and general condition of compost to be tested.
2. Set up control pots (negative control)
  - Fill **3 pots** with potting mix, tapping several times on the counter top to settle mix uniformly.
  - Label pots.
3. Prepare test pots and label pots. The mix will vary depending on garden scenario:
  - If testing soil or topsoil mix where plants grown exhibited suspicious symptoms in the previous growing season: fill **3 pots** with straight soil/topsoil mix tapping several times on the counter top to settle mix uniformly.
  - If testing compost received for the current growing season: mix 2 parts compost to 1 part plain potting mix in a clean plastic bag. (**The ratio is 2:1 by volume, compost to potting mix.**) Use separate, new 4" pots to measure compost and potting mix. Measure 2 pots of compost and 1 pot of potting mix for each compost to be tested. Fill **3 pots** with the compost blend, tapping several times on the counter top to settle blend uniformly.
4. Plant **3 seeds** in each prepared and labeled pot, pushing seeds into potting mix so they are just under the surface.

**\*\*Note:** Additional composts may be tested simultaneously using one set of controls. Prepare 3 pots for each test material. Use clean plastic bags to mix each batch to avoid cross-contamination.

5. Grow plants
  - Position pots in random order on bench **with plastic saucers underneath each one.**
  - Space pots far enough apart to avoid splashing media from one pot to the next during watering.
  - **Water each pot carefully.** Keep potting mix uniformly moist; minimize water leaching into saucer. If excess water drains into saucer, allow it to be re-absorbed back into the pot.
  - Maintain consistent growing conditions with 12 hours light, supplemented with fluorescent grow lights as necessary. Temperature should not drop below 50 F at night.
6. Evaluate plant growth
  - Record germination from each pot. In order to consider the results valid, at least two seeds in each of two pots from each replicate must germinate. (That's a total of 4 out of the nine seeds planted.)
  - Grow plants **until three sets of leaves appear**, from **14 to 21 days**, depending on growing conditions.
  - Compare plants from compost-blend pots to negative control.
  - Determine level of auxinic herbicide damage. Use Figures 1 and 2 below as a guide.

**Figure 1: Pea plants showing slight and severe damage, 3 weeks after planting.**



A. Pea plants with slight damage

Note: leaf curling



B. Pea plants with severe damage

Note: leaf curling and stunted plants

**Figure 2:** Results of a bioassay with known concentrations of aminopyralid. Plants shown are at six weeks after planting. Note leaf curling on plant grown in 5ppb (parts per billion) aminopyralid and death of plants at higher levels.

